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Microbiological Experiments Onboard CubeSats – A Review and Prospects

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Since the beginning of CubeSat operations, most missions have focused on technology demonstration, student outreach, and, to a lesser extent, on in-situ scientific research; namely, space environment characterization. Nevertheless, there have been four CubeSats with a space life sciences focus. GeneSat-1, launched in 2006, proved the feasibility of conducting microbiological experiments onboard a CubeSat. Using *E. coli* as a model organism, it successfully acquired and transmitted growth and gene expression data back to Earth. PharmaSat, launched in 2009, was designed and built to analyze yeast and antifungal treatment, and to assess drug action in space. This second mission further demonstrated that CubeSats could serve as cost-effective platforms to answer fundamental biological questions. O/OREOS followed suit in 2010, housing two independent scientific payloads and increasing the mission duration from two weeks to over six months. In 2014, SporeSat was launched as a technology demonstrator for lab-on-a-chip devices using the fern *Ceratopteris richardii* as a model organism. Although life sciences experiments onboard pico- and nano-satellites are still far from being a trend, these four NASA Ames satellites have shown that this is feasible. This paper analyzes these CubeSats from engineering and programmatic points of view. Satellite subsystems, *e.g.* power and communication, and their ground control systems are described. Operational parameters such as orbital information are also detailed. A special focus is given to the payload hardware design, development and operation. Finally, upcoming microbiology-focused CubeSats, EcAMSat and BioSentinel, are also discussed. From the engineering point of view, it is concluded that the establishment of a bus that permits interchangeable, standard payloads is recommended to streamline the microbiological research development process and duration, which in turn could potentially reduce programmatic costs. This review shows the potential of doing microbiology experiments through a CubeSat platform and the benefits they can bring to space biology research.

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1. Introduction

Life sciences research has taken place in space since the beginning of the Soviet and American space programs. Microbiological experiments, specifically, started in Korabl-Sputnik 2 and Discoverer 17 in 1960 using *E. coli* and *Clostridium sporogenes* as their model organisms, respectively [1-4]. Ever since, multiple spacecraft have been used as platforms for these types of investigations, including the Soviet Vostok, and Mir space station, and the American Gemini, Apollo, and Space Shuttle [1]. These spacecraft had their own primary mission and hosting these experiments was not their priority; in other words, experiments were designed around the spacecraft's main mission. This premise started turning around with the advent of CubeSats. The CubeSat unit standard is a $10cm^3$, around 1.3 kg cubic satellite with its own Attitude Determination and Control System (ADCS), power and communication subsystems, and are capable of carrying their own payload [5]. These satellites can be of one or more units, *e.g.* a 3 unit (3U) CubeSat is 10cmx10cmx30cm in size. CubeSats allow for the spacecraft to be designed around the experiment's goals and objectives, and not the other way around. Since the first ones in 2002, the number of CubeSats launched to lower Earth orbit (LEO) has grown exponentially.

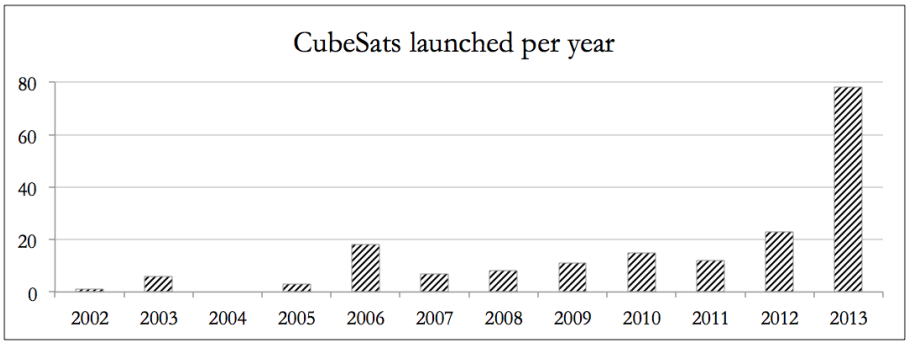

Figure 1. Growth of number of CubeSats launched since 2002.

Only four of these spacecraft have had a space life sciences payload and all of them were managed by the NASA Ames Research Center: GeneSat-1, PharmaSat, O/OREOS and SporeSat, launched in 2006, 2009, 2010 and 2014, respectively. The first three had microbiological experiments onboard, while SporeSat conducted a plant-focused experiment.

Although these missions have been reported on before, no publication analyzed them together in a systematic fashion. The references described in Table 1 can be used for further and more detailed reading about each of these missions.

CubeSat	References
GeneSat-1	[6-11]
PharmaSat	[12-16]
O/OREOS	[17-23]
SporeSat	[24]

Table 1. Publications about each of the four space life sciences CubeSats.

2. Background

NASA Ames has taken an evolutionary approach to the development of the four space life sciences CubeSats in that the technologies flown in a satellite have been used as the basis for the next one. This allows reducing risk and increasing the probability of mission success. GeneSat-1 was designed, built and flown as a technology demonstrator that *in-situ* biological research and processing in a small satellite was feasible [7]. PharmaSat was the first nanosatellite to host a competitively selected, peer-reviewed, science-driven mission. It "borrowed heavily" from GeneSat-1 in terms of the 1U bus [12]. The bus of the Organism / ORganic Exposure to Orbital Stresses (O/OREOS) satellite was an improved version of the 1U control bus of PharmaSat [19]. The latter two satellites had ground control stations and operations based on those of the first one [8,12,17]. Secondary to their scientific goals, each of these missions had a Science, Technology, Engineering and Math (STEM) out-reach objective, for which they carried amateur radio beacons [7,12,17]. This permitted people around the world to track the satellites as they orbited Earth and transmitted data.

CubeSat	Launch Date	Launch Vehicle	Launch Site
GeneSat-1	Dec 16 2006[6]	Minotaur-I[28]	NASA Wallops[6]
PharmaSat	May 19, 2009 [12]	Minotaur-I[29]	NASA Wallops[29]
O/OREOS	Nov 19, 2010 [18]	Minotaur-IV[18]	Kodiak, Alaska[17]

Table 2. Launch information for the three microbiological research CubeSats.

From a programmatic point of view, the overall project time was reduced from the first until the third satellite. GeneSat-1 took three years from idea to launch [6] while it took O/OREOS less than two years from the project kick-off meeting to delivering for launch [20].

Because not much data has yet been published about SporeSat (launched in 2014) it's review in this article is limited. It can be mentioned, however, that this satellite's bus was also based on flown technologies in PharmaSat and O/OREOS [24].

3. Mission Objectives, Requirements, and Constraints

The scientific missions of each of these CubeSats increased in complexity as technologies and operations were verified and validated with each subsequent flight. The mission objectives created specific requirements and constrains on the spacecraft's hardware and operations. For that reason, all of these parameters are analyzed together.

GeneSat-1's scientific objective was to characterize bacterial growth and metabolics using *E. coli* as the model organism. This implied growing bacteria in a suitable, controlled environment, and acquiring and transmitting data back to the scientists. These experiment objectives created the following mission requirements, constraints and drivers [7]:

1. A microfluidic-based payload needed to be capable of operations in microgravity
2. The payload temperature needed to be regulated to within 0.5ºC
3. High-quality optical sensors needed to be miniaturized for *in-situ* data acquisition
4. An amateur radio needed to be operational onboard the satellite (derived from the secondary STEM outreach mission)

The latter required the 3U form to be changed and customized to allow the radio beacon to fit. This, in turn, required the modification of the CubeSat deployer (P-POD).

PharmaSat's scientific mission objective was to study the effects of microgravity on yeast (*S. cerevisiae*) growth and metabolism, and on antifungal efficacy via three-color optical absorbance. To achieve these goals, the satellites needed to accomplish five main functions [12,13,14]:

1. Provide life support for the well plate in the payload
2. Introduce antifungal agents into the wells
3. Measure optical density in the wells to calculate population growth
4. Measure culture viability
5. Telemeter data back to Earth

O/OREOS carried two payloads, Space Environment Survivability of Life

Organisms (SESLO) and Space Environment Viability of Organics (SEVO), each with their own mission objectives. SEVO studied four organic compounds, an amino acid, a quinone, a polycyclic aromatic hydrocarbon and a metallo-porphyrin, as they were exposed to the space environment [19]. The objective of SESLO was to measure long-term survival, germination, and metabolic activity of *B. subtilis* spores exposed to microgravity and ionizing radiation for up to six months [19]. Besides gravitational regime, O/OREOS included radiation as another independent variable because of its inclined orbit, which made it travel through relatively weak regions of the magnetosphere. The estimated radiation dose rate was about 15 times the one that would be observed in an ISS-like orbit [20].

CubeSat	Inclination	Altitude	Darkness / Sunlight
GeneSat-1	40º[6]	410 km[6]	60 min / 30 min[6]
PharmaSat	40º [12]	460 km[12]	~65 min / ~32 min[14]
O/OREOS	72º [17]	650 km[17]	N.F.

Table 3. Orbital information from each satellite. O/OREOS highly inclined orbit provided a high-radiation environment. N.F. indicates that the information was not found in the surveyed literature.

Because of its high orbit, O/OREOS' natural decay back into the Earth's atmosphere would take 60 years. In order to accelerate this process, a deorbiting mechanism was included.

SporeSat investigated the gravitational threshold for calcium ion channel activation in the spores of a fern using a lab-on-a-chip approach. The objective was to acquire data to help understand the mechanisms of plant cell gravity sensing. To differentiate the role of gravitational regime from other aspects of the spaceflight environment (*e.g.* radiation), some of the samples were centrifuged to simulate 1*g* while in space. This created new requirements, constraints and drivers onto the satellite design.

4. CubeSat Subsystems

Each new satellite was based on technical heritage from the previous one, and for that reason, several components and engineering approaches were repeated from one mission to the next. On the other hand, different mission objectives called for specific engineering solutions. The following tables describe technical information for each of these satellites, categorizing them mainly by subsystem.

CubeSat	Mass	Size
GeneSat-1	4.4 kg[6]	3U*[6]
PharmaSat	<5 kg[12]	3U*[12]
O/OREOS	5.5 kg[17]	3U*[17]
SporeSat	~5.5 kg[24]	3U*[24]

Table 4. Size and mass of each of the CubeSats. 3U*: these satellites were slightly larger (10cmx10cmx34cm) than a standard 3U in order to accommodate an amateur radio [12].

CubeSat	Solar Panels	# Solar Panel Sets	Power Produced	Battery Type
GeneSat-1	Triple-junction [6]	4[6]	Max: 10.07 V, 1043 mA[8]	(1) Li-ion[6,7]
PharmaSat	Triple-junction [12]	N.F.	N.F.	(1) Li-ion [12,14]
O/OREOS	N.F.	N.F.	4-5 W[17]	Li-ion[18]

Table 5. Power subsystem. Value in parenthesis indicates number of sensors onboard the CubeSat. N.F. indicates that the information was not found in the surveyed literature.

Communications	Radio Beacon	Payload Heater	Other Payload Equipment
~225 mA	<10 mA	500 mA	<20 mA

Table 6. GeneSat-1 Current Consumption as reported in [7].

As seen in Table 6, reference [7] provides detailed information regarding current consumption by different components in the satellite. Similarly, Minelli et al. [8] reports that during the GeneSat-1 mission, there was only a 2.6%/year current degradation and no voltage degradation through time. Although similar information for the other satellites was not found, Ricco et al. [14] indicate that PharmaSat's payload heater consumed 2W of power. Beyond power consumption, the battery, with an operational range of (0°C-45°C), was the temperature-limiting component in PharmaSat [13].

The attitude determination and control system (ADCS) for the first three CubeSats was based on longitudinal magnetic rods (to align to Earth and pointing the antenna properly) and hysteresis rods (to damp "wobble") [6,12,18,19]. Beyond that, reference [19] reports that O/OREOS rotated about its long axis at ~0.5-2 RPM.

Although not originally designed for the space environment [8], the commercial-off-the-shelf (COTS) and unmodified Microhard Systems Inc. MHX-2400 was selected as GeneSat-1's transceiver [7]. This component was flown again in PharmaSat and a similar one (since the 2400 model was no longer in production) was used in O/OREOS. The communication subsystem's components are a good example of how each new satellite used technologies already proven in space by a previous mission. Finally, it is reported by Ehrenfreund et al. [20] that O/OREOS carried a 5cmx5cm patch antenna.

CubeSat	Transceiver	Frequency	Radio Beacon
GeneSat-1	MHX-2400[7]	2.4 GHz[7]	450 MHz[7]
PharmaSat	MHX-2400[12]	2.4 GHz[12]	450 MHz[12]
O/OREOS	MHX-2420[17]	2.4 GHz[17]	437 MHz[17]

Table 7. Communication subsystems components.

CubeSat	Main Antenna Size	Main Antenna Gain	Secondary Antenna Size	Secondary Antenna Gain
GeneSat-1	(1) 18 m [7]	40 dBi[8]	3 m[8]	10 dB[8]
PharmaSat	(1) 18 m [12]	N.F.	(2) 3 m [12]	N.F.
O/OREOS	N.F.	N.F.	(2) 3 m[17]	N.F.

Table 8. Ground control station details. Value in parenthesis indicates quantity. N.F. indicates that the information was not found in the surveyed literature.

According to Kitts et al. [7], there was no link degradation over the first year of GeneSat-1 operations and that S-band communications were also achieved with the secondary (3-meter) antenna. Originally done for GeneSat-1 and later used by the following missions, a secure Internet link using a data streaming architecture was put in place between ground control station and mission operation center [7]. Kitts et al. [12] report that S-Band communications were conducted the same for PharmaSat as they were with GeneSat-1. Furthermore, it indicates that the 18-meter antenna had a 0.1° accuracy and a 5 °/sec maximum azimuth rate and that two 3-meter antennas were also used for S-Band communications. The outreach radio beacon was received through a Yagi antenna with Doppler compensation [12]. Similarly, O/OREOS used GeneSat and PharmaSat heritage architecture, using two 3-meter S band dishes [17]. During the first six months of O/OREOS mission, 6 MB total science and health and status data were downloaded through one or two contacts per day, each of 5 to 15 minutes in duration [20].

Regarding computing capabilities, a PIC-based data handling board was

used both in GeneSat-1 [7] as in PharmaSat [12], and the latter had a communication rate capability of 960 bps [12]. In GeneSat-1, there were no CPU resets or single event upsets after 18 months. In the other hand, the system's clock had drifted 25 hours after 15 months [7].

Apart from the main subsystems, these CubeSats carried complete suites of sensors as described in Table 9. In the case of O/OREOS and its high orbit that would take 60 years to reenter the atmosphere, a de-orbit mechanisms was necessary. This was based on a propellantless nanokite that increased the CubeSat surface area using a 60% with a germanium-coated Kapton® film. This film was 7.5 mm thick prior to deployment and is expected to decrease the de-orbit time to 22 years [17,18].

CubeSat	Temperature	Pressure	Relative Humidity	Acceleration	Radiation
GeneSat-1	(6) Analog Devices AD590[10]	(1) Motorola MPXH6101A[10]	(1) Sensirion SHT 15[10]	(3) Silicon Designs 1221–0021[10]	Hamamatsu PIN diode, S3071 [10]
PharmaSat	(6) Analog Devices AD590[12,14]	(1) not defined[14]	(1) not defined[14]	Yes but not defined[14]	PIN diode[14]
O/OREOS	Yes but not defined [18]	Yes but not defined [18]	Yes but not defined [18]	N.F.	Yes but not defined [18]

Table 9. Other sensors in the CubeSats. Value in parenthesis indicates quantity. N.F. indicates that the information was not found in the surveyed literature.

5. Payload Hardware

Since all of these are science-driven missions, the most important subsystem is the payload itself. For all of the microbiological satellites, the core of the payload was a microfluidic card with several wells and placed inside a pressure vessel filled with humidified air to enable gas [7,14,18]. However, this is not true for SporeSat, which had a different approach.

CubeSat	Pressure vessel volume	Number of wells in well plate	Well volume
GeneSat-1	0.9 L[7]	12[6]	110 µL[6]
PharmaSat	1.2 L[14]	48[14]	100 µL [14]
O/OREOS	N.F	36[18]	75 µL [18]

5.1 GeneSat-1

The payload was based on a twelve (110 µL) well plate – two of which were solid-state controls. The plate was an acrylic manifold that connected all ten wells to an input and an output port, each with a 0.5 µL filter. One of the faces of the plate was covered with a 0.5 mm optical-quality acrylic plate and the other with a 75 µm gas permeable membrane (polystyrene). The plate was connected to a 15 mL polyethylene vinyl acetate (PEVA) bag filled with growth medium through a solenoid valve (Parker). Similarly, a second PEVA bag was connected to the plate outlet. Each of the twelve wells had a 3W blue LED to produce fluorescence excitation and a 2.3mW green LED for illumination to acquire optical density. A ratio of fluorescence (from blue LED) to scattering (from green LED) was used to quantify gene expression on a per-organism basis [6].

5.2 PharmaSat

The payload hardware was contained within a 1.2 L pressurized vessel [14], which in turn was covered in Multi-Layer Insulation (MLI) blankets (including Ultem and Delrien) and Aluminum gold plating for heat transfer optimization [13]. To reduce heat conduction, the payload was attached to the bus with Titanium bolts and Ultem washers. The payload components were warmed with flexible Kapton heaters. These were two, 2 Watt Minco aluminum thermal spreader heaters, one on each side of the fluidics card with holes in each well [13]. They were capable of providing a temperature stability of less than 0.3 ºC. The heaters consumed 2W in average to maintain the fluidics card at 27ºC. The 10cmx20cm fluidics card was made of laser-cut poly(methylmethacrylate) layers and included forty-eight 100 µL wells (4 mm diameter and 7.8 mm deep) and 11 solid-state reference wells. It had four independent sets of manifolds with 1.2 µm nylon fiber membrane filters. There was a 51 µm polystyrene gas-permeable membranes on each side of the fluidics card [14]. The payload hardware also included a miniaturized environmental control system, microfluidics systems with pumps, valves and a series of optical sensors [12].

5.3 O/OREOS

The SESLO payload was 1U in size and had three Bioblocks. Each block had twelve 75 µL wells interconnected through microfluidic channels and a solenoid valve to a reservoir with germination medium colored with Alamar blue. Each block was to be used to independently assess growth at three different times: 14, 97, and 180 days. Three different wavelengths where use to acquire data: 450, 525, and 615nm. As Alamar blue reacts with metabolites it changes from blue to pink and then from pink to colorless [18].

5.4 SporeSat

SporeSat used three lab-on-a-chip devices called BioCDs. The sensors allowed for real-time measurement of calcium signaling. Two of these chips were centrifuged and the third one remained in microgravity [24].

6. Prospects

Currently NASA Ames has two more CubeSats with microbiological research mission under development: EcAMSat and BioSentinel, both 6U in size and manifested to launch in 2015 and 2017, respectively [26,27]. EcAMSat's mission is to investigate the effects of microgravity on the dose-dependent antibiotic response and resistance of *E. coli* [25]. BioSentinel is scheduled for launch in late 2017 on board NASA Space launch system (SLS) exploration mission 1. BioSentinel will be deployed from the launch vehicle upper stage, putting it on a lunar flyby (~700km) trajectory and eventually into a heliocentric orbit. During its 12 to 18 month mission it will measure the damage and repair of DNA in yeast and compare it to data from onboard physical radiation sensors [27].

7. Discussion

GeneSat-1 proved the feasibility of flying microorganisms to space and conducting in-situ data acquisition to monitor growth and gene expression. Approximately 60% of the power demand (~500 mA) came from the heater, to maintain 34ºC. This is can be expected to be a frequent driver for life sciences research conducted in small satellites. GeneSat-1 proved to be successful in environmental control, bus performance and on the biological experiment. This success was repeated with PharmaSat.

O/OREOS increased the mission duration from two weeks to over six months. According to the publications reporting about it, the SESLO experiment was successful under all engineering criteria, which reduces risk for future hardware development. O/OREOS operation in a high-radiation environment validated technologies that can be used by CubeSats venturing beyond LEO.

The upcoming BioSentinel mission will include deep space communications and navigation, autonomous attitude control and momentum management, a propulsion system and will go beyond LEO.

Since CubeSats are secondary payloads there are constraints placed upon them such as lack of access for late loading or lack of power for thermal con-

trol prior to launch. Experiment automation produces constraints as well as benefits: it creates engineering requirements on the payload data acquisition system but it avoids manifesting astronaut operation time and avoids the need for sample return. Some of the benefits of flying life sciences experiment in CubeSats may include reduced cost, risk and turnaround time compared to more complex and not-dedicated platforms. As demonstrated with GeneSat-1, PharmaSat and O/OREOS, the use of a standard bus that permits interchangeable payloads helps streamline the microbiological research development process and duration, which in turn can potentially reduce programmatic costs.

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